Hairy Canola Deters Flea Beetle Feeding

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Abstract

Flea beetle, chiefly *Phyllotreta crucifrerae*, behaviour and feeding on lines of *Brassica napus* canola with elevated levels of trichomes on true leaves were compared with feeding on leaves of parent seedlings. The beetles became agitated and seldom settled down to feed when exposed to the hairy seedlings. Levels of feeding on the new lines were measured in a laboratory trial at Saskatoon, and in field trials at Saskatoon (2005, 2006) and Lethbridge (2006). Initially, feeding levels were lower on cotyledons of the recombinants than on those of the parental line, possibly because of the increased angle of recombinant cotyledons relative to the soil surface. As cotyledons became more horizontal on recombinant seedlings, feeding upon them increased, but feeding on true leaves of the transgenic lines was less than on true leaves of parental lines The decreased feeding on true leaves of recombinant seedlings is a first step in developing canola resistance to the insect.

Introduction

Crucifer-feeding flea beetles are the most prevalent and economically costly insect pests of canola on the North American Great Plains, with annual losses, even in non-outbreak years, averaging over $200 million (Knodel and Olsen 2002). Severe flea beetle (*Phyllotreta* spp.) feeding at the very early stages of canola development can result in seedling mortality, delayed maturity and yield losses (Lamb 1984). Currently, the principal means of flea beetle management is by the use of insecticides. Of the 5.5 million ha of canola seeded annually in Canada, over 90% is planted using seed coated with an insecticidal seed treatment. In many locations flea beetle infestation levels do not warrant the use of the insecticide, but it is seen by producers as insurance against potential flea beetle damage. With the de-registration of insecticides traditionally used for flea beetle control and their replacement with more specific but more costly ones, an alternative to insecticidal control that is less damaging economically and environmentally while being effective and easy to use is urgently needed. Such an alternative is host plant resistance.

Trichomes, or leaf hairs, are one type of defense that plants employ to combat stresses such as herbivory. Dense coverage of trichomes on mature leaves of *Brassica villosa*, a Mediterranean wild Brassica species, protect it from feeding by flea beetles (Palaniswamy and Bodnaryk, 1994), while trichomes on the pods of mustard *Sinapis alba* protect them from similar feeding (Lamb, 1980). Cotyledons and first true leaves of canola *B. rapa* and *B. napus* cultivars, which are the

most vulnerable to feeding by flea beetles, are virtually hairless. When *B. napus* var. Westar was transformed by the introduction of two genes from the related species *Arabidopsis thaliana*, *bHLH* gene 35s:*GLABRA3* (*GL3*) and *MYB* gene *GLABRA1 (GL1)*, the density of trichomes on the first three true leaves increased from fewer than 1 per cm^2 to over 1000 (Gruber et al. 2006). This increase in leaf hairs give the transformed seedlings a furry, pincushion-like appearance. However, because the genes are not expressed on maternal tissue, the cotyledons of these transformed plants remain hairless. On the other hand, orientation of the newly expanding cotyledons of the transgenes is somewhat more vertical than that of the Westar seedlings. Our objective in the current project was to determine if feeding by flea beetles is decreased on the transformed canola seedlings.

Materials and Methods

Resistance of hairy seedlings to flea beetles was examined in three ways: by comparison of feeding behaviour of individual flea beetles on hairy and parental canola seedlings, by laboratory choice bioassays of hairy and parental lines, and in field tests of hairy lines with parental lines and commercial cultivars. Observations of pre-feeding behaviour on Westar and Hairy canola were undertaken after the manner of Henderson et al. (2004), in which the actions of single beetles were filmed until prolonged feeding, > 100 seconds, was followed by a rest period of more than 2 minutes. Although many flea beetles were utilized, only about 30 of the ones paced in the hairy canola test arenas exhibited behavour typical of unconfined beetles in the wild. Of these, only six settled down enough to have their movements filmed and analyzed.

To screen crucifer seedlings for antixenosis resistance to flea beetles in the laboratory, a 1x1x0.25 m plexiglass arena with a Styrofoam base holding 32 plastic vials of one seedling each, 16 replicates of two entries, was used (Palaniswamy et al. 1992). The material tested was the parent control *B. napus* cv Westar, and the "Hairy" transgene, the *GL3* single insertion transgene. Flea beetles, primarily *Phyllotreta cruciferae* (Goeze) were collected from the field, placed in holding cages at 20-22°C 16 hr per day, 15-16°C 8 hr night conditions, and fed a water solution and cabbage leaves, until starved for 24 hrs prior to testing. Approximately 10 flea beetles per seedling were placed in each of three arenas, and the percentage of flea beetle feeding on cotyledons was assessed at timed intervals of 6, 24, and 48 hours. Foliar damage was estimated as the percent leaf area eaten of the cotyledons and first true leaves, measured in increments of 10, from 0, not fed upon, to 100, cotyledon or leaf entirely destroyed.

Field trials were undertaken a the Research Farm of Saskatoon Research Centre, on the outskirts of Saskatoon in 2005 and 2006, and at the Lethbridge Research Centre in Lethbridge, AB, in 2006. Trials were comprised of four replicates planted in three- or four-row plots 6.1 m in length with 0.30 m row spacings, using a randomized complete block design. In 2005 entries included two sources of the Westar parent, one from Saskatoon and one from the Alberta Research Council in Vegreville, the Westar lines treated with thiamethoxam seed coating insecticide (10.3% thiamethoxam at 375 ml per 25 kg seed), commercial cultivar 45A65, and two *GL3* hairy lines. In 2006 the less promising of the two *GL3* transgenes was dropped and the commercial cultivar H21 added. As well, thiamethoxam treated seed of 45A65 and H21 were included in the 2006 trials, while the seed-treated Westar from Alberta Research council was dropped.

Results and Discussion

In the feeding behaviour trial, flea beetles on Hairy canola appeared agitated and unsettled, and had difficulty reaching the surface of the cotyledon. Of the pre-feeding time that flea beetles were on Westar seedlings, 48.5 % was spent in direct contact of the mouth with the cotyledon, while flea beetles on Hairy canola touched the cotyledon with their mouth only 39.6% of the time. Beetles on Hairy canola spent 20.9% of their time running around the cotyledon, while those on Westar cotyledons ran for 7.7% of their time. On flea beetle on Hairy canola was observed attempting to feed while in a vertical position, standing on his head so to speak.

In the laboratory bioassay, flea beetles avoided feeding on the hairy seedlings. Both cotyledons and first true leaves of the Westar parent line had more feeding than did Hairy seedlings (Table 1). This reluctance to feed was mirrored in the number of flea beetles found on each canola line, with Westar seedlings having more than twice as many flea beetles on them as did the trichome-enriched transgene plants

Table 1. Feeding damage levels on cotyledons and first true leaves of *Brassica napus* canola cv Westar and a Westar-transformed line with elevated numbers of trichomes on stems and leaves, as well as the number of flea beetles on seedlings of the two plant lines 48 hr after flea beetle placement in the bioassay arenas.

	% Tissue area eaten after 48 hrs			
	Cotyledons	1st true leaves	Both tissues	No. beetles plant^{-1}
Westar	15.0 a*	30.1 a	21.7a	5.7 a
Hairy	6.0 b	23.1a	16.6b	2.7 b
P≤	0.007	0.076	0.004	0.0001

* values followed by the same letter are not significantly different at P ≤0.05, ANOVA and Tukey's standardized range test.

In the Saskatoon 2005 field trial flea beetle feeding on cotyledons was light on all entries, varying from 0.2 to 9.5%. However, on first and second true leaves the two transgenic entries had feeding levels that were lower than those of their parental counterpart, and that were statistically similar to the levels on insecticide treated controls (% leaf area eaten on first and second true leaves 12 days after emergence - Westar ARC - 31.8 a, Westar - 24.4 ab, 45A65 - 21.9 ab, GL3 - 15.6 bc, Hairy - 15.3 bc, Westar insecticide treated - 11.7 c, Westar ARC insecticide treated - 10.4 c; values followed by the same level are not significantly different, ANOVA and Tukey's Range test at P≤0.05).

In 2006 at Saskatoon, flea beetle feeding was the lowest on Hairy cotyledons shortly after emergence (Fig. 1, top left). As true leaves emerged, flea beetles moved to these tissues on untransformed plants, but remained feeding on Hairy canola cotyledons (Fig. 1, top row right,

middle row, left). Damage on the true leaves of Hairy canola remained low as they grew, remaining lower than on the untrasnformed parental material (Figure 1 middle row right, bottom graph).

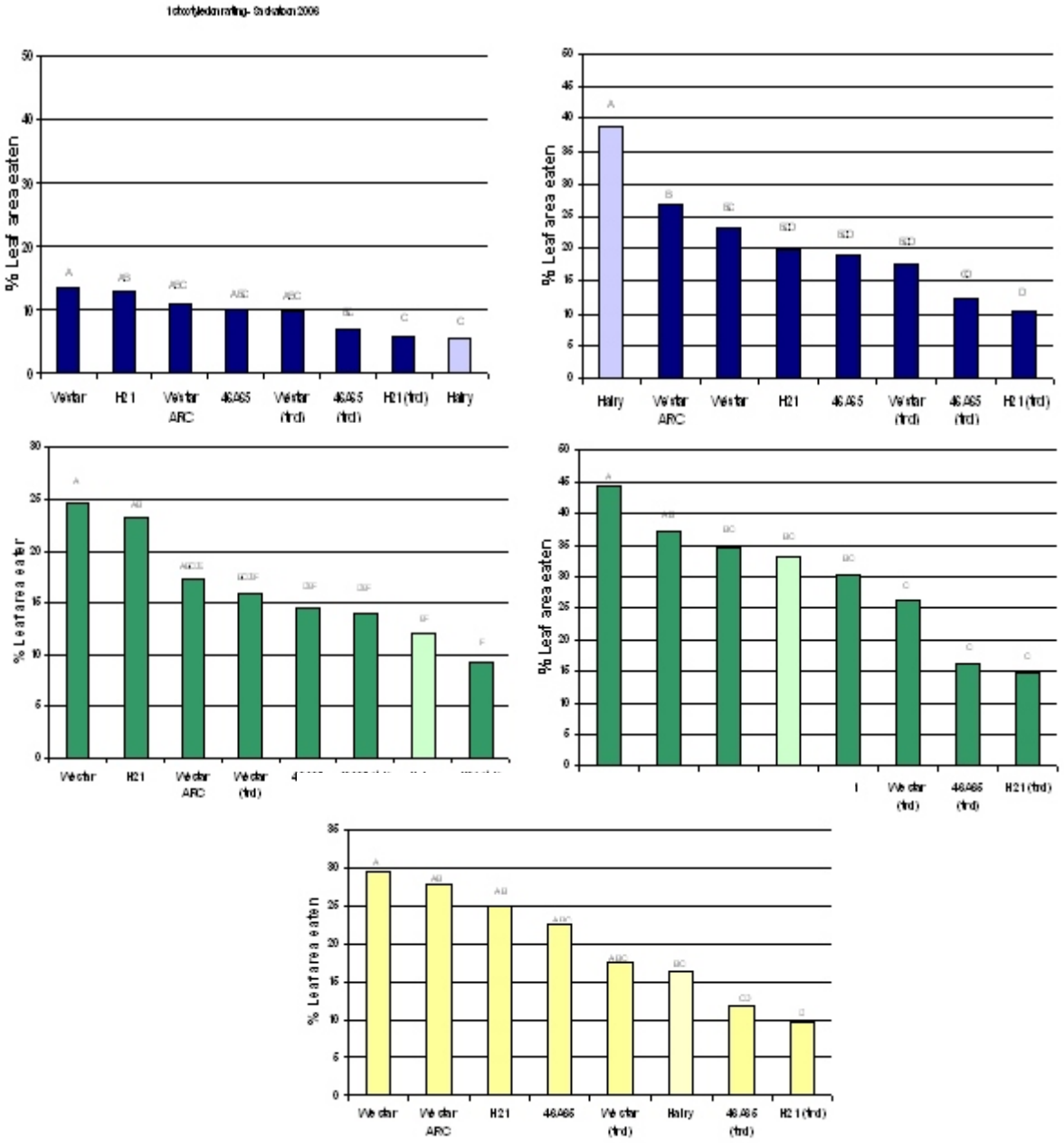

Figure 1. Flea beetle damage to seedlings of canola *Brassica napus* lines at Saskatoon, 2005. Top graphs: cotyledon damage at left) 6 and right) 14 days after emergence; middle row: damage to first and second leaves left) 6 and right) 14 days after emergence; bottom graph: damage to third and fourth true leaves 14 days after emergence.

At Lethbridge in 2006 absolute flea beetle feeding levels were higher than at Saskatoon, but the

patterns of feeding were similar to those seen in this province (Figure 2). Initial feeding levels on cotyledons were low (data not presented), but increased (Fig. 2 top left) as true leaves emerged, which were fed upon minimally (Fig. 2 top right). Third and fourth true leaves had feeding levels comparable to entries sown with insecticide-treated seed (Fig. 2 bottom).

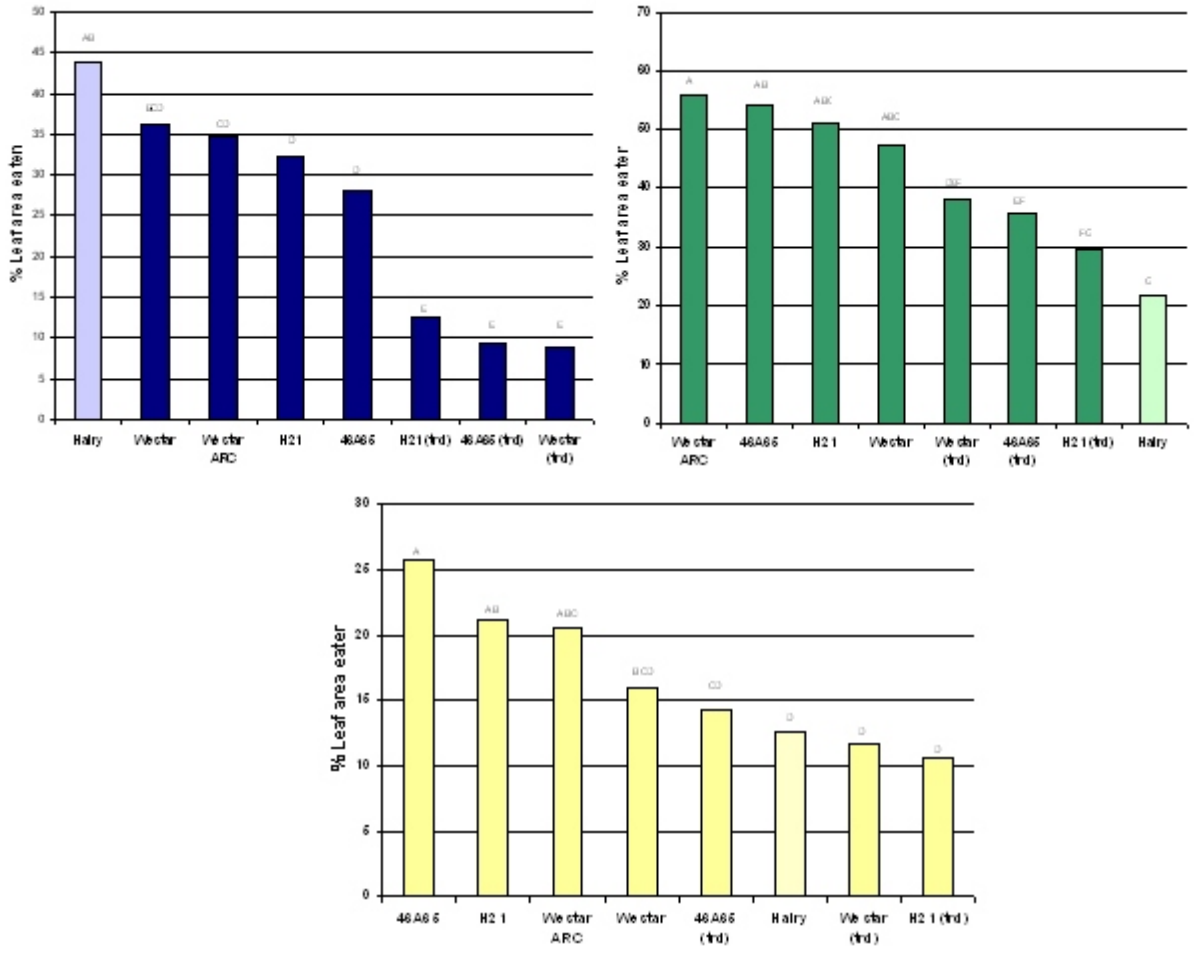

Figure 2. Flea beetle damage to seedlings of canola *Brassica napus* lines at Lethbridge, 2006. Top left: cotyledon damage at 12 days after emergence; top right: damage to first and second leaves 20 days after emergence; bottom graph, damage to third and fourth true leaves 20 days after emergence.

Conclusions

Transgenic *Brassica napus* canola lines with elevated levels of trichomes on developing leaves were tested in a laboratory trial at Saskatoon, and field trials at Saskatoon and Lethbridge. Flea beetles, *Phyllotreta* spp., exhibited abnormal behaviour when confronted with hairy seedlings, and preferred not to feed on them. This feeding deterrence is a first step in developing crop resistance to the insect.

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